

A14 Induction test 28/07/09

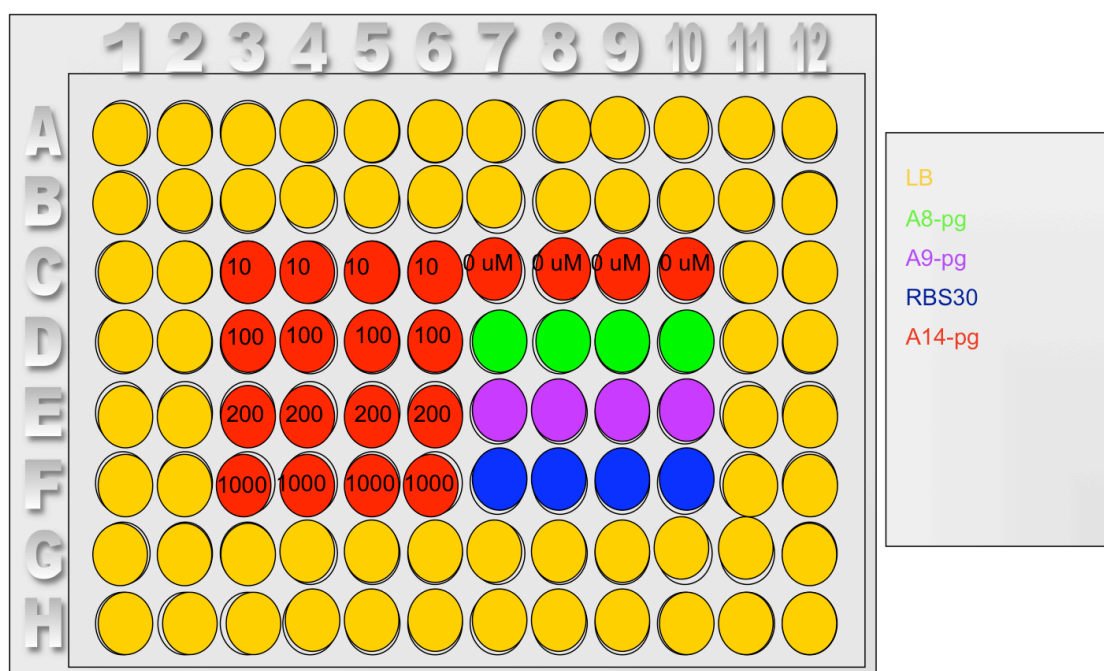
Description: Induction test of A14 with different concentration of IPTG

Purpose: Induction test of A14 with IPTG at different concentrations. Positive controls: A8 and A9. Negative control: RBS30.

Methods: A flat-bottom non sterile plate is used. 96 wells are filled with:

- 200 ul LB+Amp,
- 200 ul bacterial cultures incubated overnight 37°C 220 rpm and then diluted 1:1000 and incubated for further 5 hours.

We want to start from a normalized OD of 0.025 and arrive to 0.17 (see Barry Canton).



The induction is in uM

Protocol:

- The plate is filled as described in Methods
- The instrument temperature is set at 37°C
- We want to see the induction in exponential phase (about 3 hours, from OD 0.05 to OD 0.15 already normalized for blank), so we sample every 3 minutes.
 - For 7 hours kinetic cycle of 3 minutes in wich:
 - 15 seconds shaking linear 3mm
 - 10 s wait
 - Absorbance and green fluorescence measure: -> GAIN SETTING: MANUAL 50
 - For the rest of experiment (untill 24 hours)

- Kinetic cycle: 20 minutes
 - Shaking 15 s linear 3mm
 - Wait 10 seconds
 - Absorbance measurement
 - GFP measurement: -> GAIN SETTING: MANUAL 50
- For a problem in software programming the sample time isn't the desired one, so we use collected data to appreciate induction at steady-state, and we decide to repeat the experiment next week to perform the desired sampling.